Asthma :- kinetics study K3 immunized

RM134L :- $\frac{400}{2000} \times 8000 = 1600 + 6400$ first challenged.

EXHIBIT G

DAY0 :-

BAL cell count (300µl). 2

C_1 :- $8 \times 10^4 = 0.8 \times 10^5$ cells.

C_2 :- $13 \times 10^4 = 1.3 \times 10^5$ cells.

B_1 :- $9 \times 10^4 = 0.9 \times 10^5$ cells.

A_2 :- $12 \times 10^4 = 1.2 \times 10^5$ cells.

B_1 :- $11 \times 10^4 = 1.1 \times 10^5$ cells.

B_2 :- $8 \times 10^4 = 0.8 \times 10^5$ cells.

C = control i.e no Alum immunized

A = immunized with Alum only

B = " " Alum-OVA

9000

12000

tissue cell count:-

		Total / 2 mice	Total volume ÷ # in Total / mice
2 mice	C :- LN 50µl = $728 \times 2 \times 10^4$ =	1.456×10^7 cells/ml	$\times 0.5/2 = 3.64 \times 10^6$
2 mice	(10ml) LUNG = $57 \times 5 \times 10^4$ =	2.86×10^6 cells/ml	$\times 10/2 = 1.425 \times 10^8$
"	(10ml) Spleen. = $448 \times 5 \times 10^4$ =	2.240×10^7 cells/ml.	$\times 10/2 = 1.12 \times 10^8$
2 mice	A :- LN 50µl = $629 \times 2 \times 10^4$ =	1.256×10^7 cells/ml.	$\times 0.5/2 = 3.14 \times 10^6$
"	(10ml) LUNG = $71 \times 5 \times 10^4$ =	3.55×10^6 cells/ml.	$\times 10/2 = 1.775 \times 10^8$
"	(10ml) Spleen = $507 \times 5 \times 10^4$ =	2.535×10^7 cells/ml.	$\times 10/2 = 1.27 \times 10^8$
2 mice	B :- LN 50µl = $849 \times 2 \times 10^4$ =	1.696×10^7 cells/ml.	$\times 0.5/2 = 4.25 \times 10^6$
"	(10ml) LUNG = $59 \times 5 \times 10^4$ =	2.95×10^6 cells/ml.	$\times 10/2 = 1.48 \times$
"	(10ml) Spleen = $391 \times 5 \times 10^4$ =	1.955×10^7 cells/ml.	$\times 10/2 = 9.78 \times$

1st α OX40L → 2nd. CD11b+ Step

DAY ①. DAY 0.

RM1342 :- 12 mice → 6ml make up 7ml.

stock = 2mg/ml. $\frac{400}{2000} \times 7000 = 1400$ (1500 + 5500).

Collagenase: $\frac{5mg}{100} \times 3000 = 150\mu l \times 2 = 300\mu l$

DNase :- $\frac{0.1}{10} \times 3000 = 30\mu l \rightarrow 40\mu l$

BAL cell count : (300µl).

A = Alum only

B = Alum - OVA.

C = Alum - OVA / RM134L

* FACS.

* $A_1 = 10 \times 10^4 = 1 \times 10^5$
* $A_2 = 6 \times 10^4 = 0.6 \times 10^5$
* $B_1 = 21 \times 10^4 = 2.1 \times 10^5$
* $B_2 = 33 \times 10^4 = 3.3 \times 10^5$
* $B_3 = 22 \times 10^4 = 2.2 \times 10^5$
* $B_4 = 10 \times 10^4 = 1 \times 10^5$
* $C_1 = 18 \times 10^4 = 1.1 \times 10^5$
* $C_2 = 8 \times 10^4 = 0.8 \times 10^5$
* $C_3 = 9 \times 10^4 = 0.9 \times 10^5$
* $C_4 = 4 \times 10^4 = 0.4 \times 10^5$

Tissue count :- Day (21)

				Total volume	Total/2 mice.	Total/mou
2 mice/gp	A	LN (1ml)	= 238 X 5 X 10^4 =		1.19 X 10^7 cells X 1/2 =	5.95 X 10^6
"		LUNG (10ml)	= 54 X 5 X 10^4 =		2.70 X 10^6 cells X 10/2 =	1.350 X 10^6
"		spleen (10ml)	= 169 X 10 X 10^4 =		1.69 X 10^7 cells X 10/2 =	8.450 X 10^6
"	B	LN (1ml)	= 358 X 5 X 10^4 =		1.790 X 10^7 cells X 1/2 =	8.95 X 10^6
"		LUNG (10ml)	= 83 X 5 X 10^4 =		4.15 X 10^6 cells X 10/2 =	2.075 X 10^6
"		spleen (10ml)	= 175 X 10 X 10^4 =		1.750 X 10^7 cells X 10/2 =	8.750 X 10^6
"	C	LN (1ml)	= 216 X 5 X 10^4 =		1.080 X 10^7 cells X 1/2 =	5.40 X 10^6
"		LUNG (10ml)	= 46 X 5 X 10^4 =		2.30 X 10^6 cells X 10/2 =	1.150 X 10^6
"		spleen (10ml)	= 203 X 10 X 10^4 =		2.030 X 10^7 cells X 10/2 =	1.015 X 10^7

FACS staining :- 0X40L : 40 well 50µl/well. 2ml + 40µl α ox Biot

~~CD11D~~ B220 } 1400µl + 25µl. + 4 chan 60
~~B220~~ CD11D } 1400µl.
CD11E } 1400µl.

DAY 2 :- K3 study.

RM134L:- $\frac{400}{2000} \times 5000 = 1000 + 400$

BAL cell count (30µl).

autoxi LUNG:

FACS * A1 = $14 \times 10^4 = 1.9 \times 10^5$

* * A2 = $9 \times 10^4 = 0.9 \times 10^5$

* B1 = $70 \times 10^4 = 7.0 \times 10^5$

* * B2 = $261 \times 10^4 = 2.61 \times 10^6$

* * B3 = $59 \times 10^4 = 5.9 \times 10^5$

* * B4 = $356 \times 10^4 = 3.56 \times 10^6$

* * C_1 = $7 \times 10^4 = 0.7 \times 10^5$

* * C_2 = $8 \times 10^4 = 0.8 \times 10^5$

FACS C_3 = $5 \times 10^4 = 0.5 \times 10^5$

FACS * C_4 = $50 \times 10^4 = 5.0 \times 10^5$.

Tissue cell count:-

Total volume / dilution factor

			total / 2 min	total / min
A	LN (1ml) 1:4	= $101 \times 4 \times 10^4$	= $4.04 \times 10^6 \times 1/2$	= 2.02×10^6 ce
	LUNG (10ml) 1:5	= $70 \times 5 \times 10^4$	= $3.50 \times 10^6 \times 10/2$	= 1.750×10^7 ce
	Spleen (10ml) 1:10	= $133 \times 10 \times 10^4$	= $1.330 \times 10^7 \times 10/2$	= 6.650×10^7 ce
B	LN (1ml)	= $685 \times 4 \times 10^4$	= $2.740 \times 10^7 \times 1/2$	= 1.370×10^7 c
	LUNG (10ml)	= $135 \times 5 \times 10^4$	= $675 \times 10^6 \times 10/2$	= 3.375×10^7
	Spleen (10ml)	= $191 \times 10 \times 10^4$	= $1.910 \times 10^7 \times 10/2$	= 9.05×10^7
C	LN (1ml)	= $325 \times 4 \times 10^4$	= $1.300 \times 10^7 \times 1/2$	= 6.50×10^6 c ~45
	LUNG (10ml)	= $68 \times 5 \times 10^4$	= $3.40 \times 10^6 \times 10/2$	= 1.700×10^7
	Spleen (10ml)	= $234 \times 10 \times 10^4$	= $2.340 \times 10^7 \times 10/2$	= 1.17×10^8 c

DAY (3) — K3 study:-

MM3/L: $\frac{400}{2000} \times 2500 = 500\,\mu l$

BAL cell counts :- A (300 µl).

LUNG	LUNG			
	*	A_1 :- ~~14×10⁴~~	$19 \times 2 \times 10^4$	$= 3.8 \times 10^5$ cells.
*	*	A_2 :- ~~20×10⁴~~	$20 \times 2 \times 10^4$	$= 4.0 \times 10^5$ cells.
*	*	B_1 = ~~295×10⁴~~	$= 295 \times 2 \times 10^4$	$= 590 \times 10^6$ cells.
*		B_2 = ~~30×10⁴~~	$= 30 \times 2 \times 10^4$	$= 6.0 \times 10^5$ cells.
*	*	B_3 =	$= 295 \times 2 \times 10^4$	$= 5.90 \times 10^6$ cells.
*	*	B_4 =	$= 200 \times 2 \times 10^4$	$= 400 \times 10^6$ cells.
*	*	C_1 =	$= 139 \times 2 \times 10^4$	$= 178 \times 10^6$ cells.
*	*	C_2 =	$= 68 \times 2 \times 10^4$	$= 136 \times 10^6$ cells.
*	*	C_3 =	$= 8 \times 2 \times 10^4$	$= 1.6 \times 10^5$ cells.
*	*	C_4 =	$= 28 \times 2 \times 10^4$	$= 5.6 \times 10^5$ cells.

tissue cell count:-

			total/2 mins	(total volume / # mins)	total/min
A	LN (1ml) 1:5	$= 151 \times 5 \times 10^4$	$= 7.55 \times 10^6$	$\times 1/2$	$= 3.78 \times 10^6$
	LUNG (10 ml) 1:5	$= 97 \times 5 \times 10^4$	$= 4.35 \times 10^6$	$\times 10/2$	$= 2.175 \times 10^7$
	Spleen (10ml) 1:10	$= 99 \times 10 \times 10^4$	$= 9.90 \times 10^6$	$\times 10/2$	$= 4.950 \times 10$
B	LN (1ml)	$= 808 \times 5 \times 10^4$	$= 4.040 \times 10^7$	$\times 1/2$	$= 2.020 \times 10$
	LUNG (10ml)	$= 158 \times 5 \times 10^4$	$= 7.90 \times 10^6$	$\times 10/2$	$= 3.950 \times 1$
	Spleen (10ml)	$= 166 \times 10 \times 10^4$	$= 1.66 \times 10^7$	$\times 10/2$	$= 8.300 \times 1$
C	LN (1ml)	$= 239 \times 5 \times 10^4$	$= 1.195 \times 10^7$	$\times 1/2$	$= 5.98 \times 10$
	LUNG (10ml)	$= 49 \times 5 \times 10^4$	$= 2.45 \times 10^6$	$\times 10/2$	$= 1.225 \times 1$
	Spleen (10ml)	$= 24 \times 10 \times 10^4$	$= 2.240 \times 10^7$	$\times 10/2$	$= 1.120 \times 1$

DAY 4

BAL cell count (300ul).

3 wks i.v.N.

* * $A_1 = 10 \times 10^4 = 1.0 \times 10^5$ cells.

* * $A_2 = 12 \times 10^4 = 1.2 \times 10^5$ cells

* * * $B_1 = 576 \times 2 \times 10^4 = 1.152 \times 10^7$ cells.

$B_2 = 30 \times 2 \times 10^4 = 6.0 \times 10^5$ cells.

* * $B_3 = 140 \times 5 \times 10^4 = 7.00 \times 10^6$ cells.

* * * $B_4 = 166 \times 5 \times 10^4 = 8.30 \times 10^6$ cells.

* * * $C_1 = 97 \times 2 \times 10^4 = 1.94 \times 10^6$ cells.

* * * $C_2 = 108 \times 2 \times 10^4 = 2.16 \times 10^6$ cells.

* $C_3 = 20 \times 2 \times 10^4 = 4.0 \times 10^5$ cells.

* * $C_4 = 5 \times 2 \times 10^4 = 1.0 \times 10^5$ cells.

tissue cell count

		Total / 2 mice	Total / 1 mice	total / 1
A	LN (1ml) 1:5 = $235 \times 5 \times 10^4$	$= 1.175 \times 10^7 \times 1/2$		$= 5.88 \times 10^6$
	Lung (10ml) 1:5 = ~~$252 \times 5 \times 10^4$~~ $104 \times 5 \times 10^4$	$= 5.20 \times 10^6 \times 10/2$		$= 2.60 \times 10^7$
	Spleen (10ml) = ~~$43 \times 10 \times 10^4$~~ $96 \times 10 \times 10^4$	$= 8.60 \times 10^6 \times 10/2$		$= 4.30 \times 10^7$
B	LN (1ml) 1:10 N.B. $\downarrow$ $437 \times 10 \times 10^4$	$= 4.370 \times 10^7 \times 1/2$		$= 2.185 \times 10^7$
	Lung (10ml) 1:5 = ~~$377 \times 5 \times 10^4$~~ $191 \times 5 \times 10^4$	$= 9.55 \times 10^6 \times 10/2$		$= 4.775 \times 10^7$
	Spleen (10ml) = ~~$98 \times 10 \times 10^4$~~ $135 \times 10 \times 10^4$	$= 1.350 \times 10^7 \times 10/2$		$= 6.750 \times 10^7$
C	LN (1ml) 1:5 = $302 \times 5 \times 10^4$	$= 1.510 \times 10^7 \times 1/2$		$= 7.55 \times 10^6$
	Lung (10ml) 1:5 = ~~$361 \times 5 \times 10^4$~~ $211 \times 5 \times 10^4$	$= 4.05 \times 10^7 \times 10/2$		$= 2.025 \times 10^7$
	Spleen (10ml) = ~~$74 \times 10 \times 10^4$~~ $106 \times 10 \times 10^4$	$= 1.060 \times 10^7 \times 10/2$		$= 5.300 \times 10^7$

FACS

1. A LN @ CD4/0X40 DAY 0.
2. B
3. C
4. A LUNG
5. B
6. C
7. A Spleen.
8. B
9. C
10. A BALF.
11. B
12. C
13. A LN CD4/0X40 DAY 1
14. B
15. C
16. A LUNG
17. B
18. C
19. A Spleen
20. B
21. C
22. A BALF
23. B
24. C
25. A LN CD4/0X40 DAY 2
26. B
27. C
28. A LUNG.
29. B
30. C

31. A Spleen CD4/0X40 DAY 2

32. B

33. C

34. A BALF.

35. B

36. C

37. A LN CD4/0X40 DAY 3

38. B

39. C

40. A LUNG

41. B

42. C

43. A Spleen

44. B

45. C

46. A BALF.

47. B

48. C

49. A LN CD4/0X40 DAY 4.

50. B

51. C

52. A LUNG

53. B

54. C

55. A Spleen.

56. B

57. C

58. A BALF.

59. B

60. C

61.

61 A LN B220/0X40L DAY0
62 B
63 C
64 A LUNG
65 B
66 C
67 A Spleen
68 B
69 C
70 A BAL Fluid
71 B
72 C
73 A LN B220/0X40L DAY1
74 B
75 C
76 A LUUNG
77 B
78 C
79 A Spleen
80 B
81 C
82 A BAL Fluid
93 B
84 C
85 A LN B220/0X40L DAY2
86 B
87 C
88 A LUNG.
89 B
90 C
91

91 A spleen B220/0X40L DAY 2.
92 B
93 C
94 A BALF
95 B
96 C
97 A LN B220/0X40L DAY 3
98 B
99 C
100 A LUNG
101 B
102 C
103 A spleen.
104 B
105 C
106 A BALF.
107 B
108 C
109 A LN B220/0X40L DAY 4.
110 B
111 C
112 A LUNG
113 B
114 C
115 A spleen.
116 B
117 C
118 A BALF.
119 B
120 C

121. A LN CD11b/OX42 DAY 0.

122. B

123. C

124. A LUNG

125. B

126. C

127. A Spleen

128. B

129. C

130. A BALF

131. B

132. C

133. A LN CD11b/OX42 DAY 1

134. B

135. C

136. A LUNG

137. B

138. C

139. A Spleen

140. B

141. C

142. A BALF.

143. B

144. C

145. A LN CD11b/OX42 DAY 2

146. B

147. C

148. A LUNG

149. B

150. C

151.

151. A Spleen CD11b/OX40L DAY2.

152. B

153. C

154. A BALF

155. B

156. C

157. A LN CD11b/OX40L DAY3

158. B

159. C

160. A LUNG ~~CD11b/OX~~

161. B

162. C

163. A Spleen.

164. B

165. C

166. A BALF.

167. B

168. C

169. A LN CD11b/OX40L DAY4

~~170~~. B

171. C

172. A LUNG

173. B

174. C

175. A Spleen.

~~176~~. B

177. C

178. A BALF.

179. B

180. C

~~181~~.

181. A LN CD11C/0X40L DAY 0.
182. B
183. C
184. A LUNG
185. B
186. C
187. A Spleen
188. B
189. C
190. A BALF.
191. B
192. C
193. A LNK CD11C/0X40L DAY 1
194. B
195. C
196. A LUNG
197. B
198. C
199. A Spleen
200. B
201. C
202. A BALF.
203. B
204. C
205. A LN CD11C/0X40L DAY 2.
206. B
207. C
208. A LUNG.
209. B
210. C
211.

211. A Spleen. CD11c /0X40L DAY 2.

212. B

213. C

214. A BALF.

215. B

216. C

217. A LN CD11c /0X40L DAY 3

218. B

219. C

220 A CLNC

221. B

222 C

223 A Spleen.

224 B

225 C

226 A BALF.

227 B

228 C

229 A LN CD4L/0X40L DA 4

230 B

231 C

232 A

233. B LAGNC

234 C

235 A

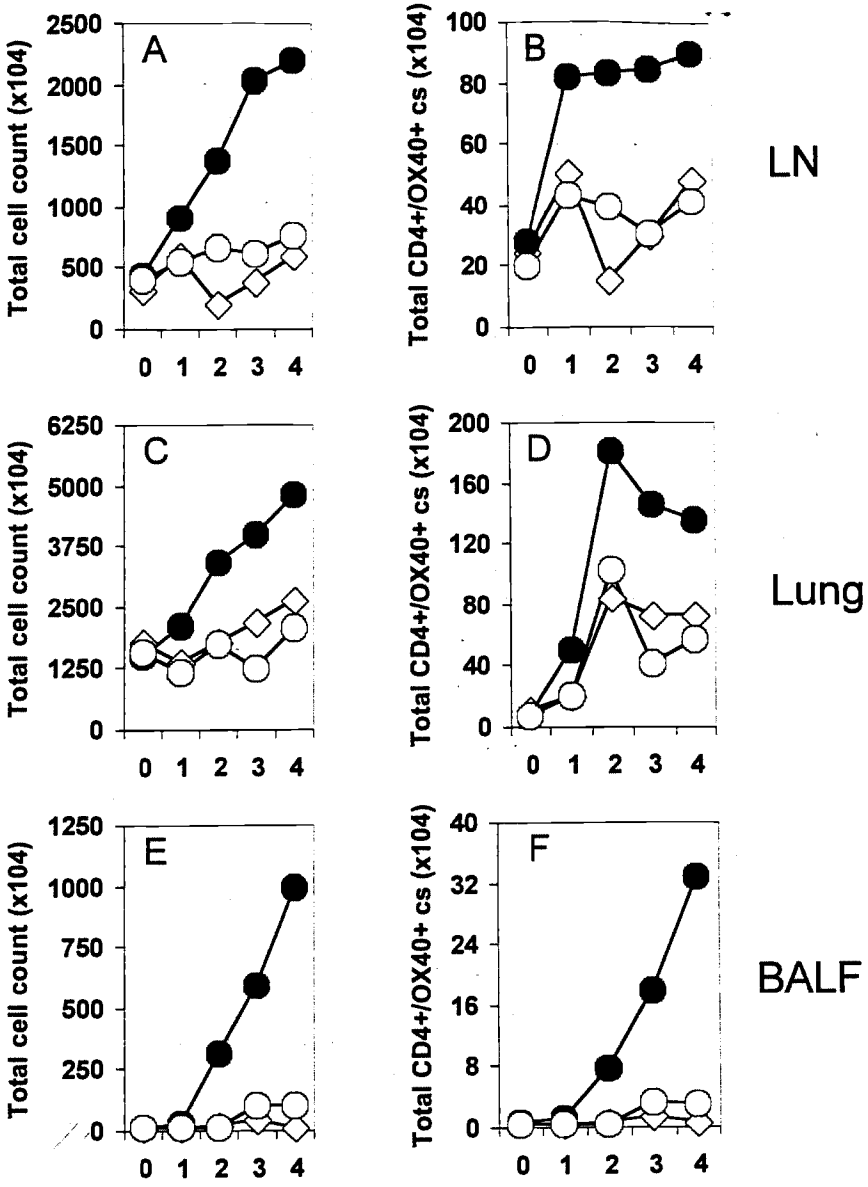
236 B Spleen.

237 C

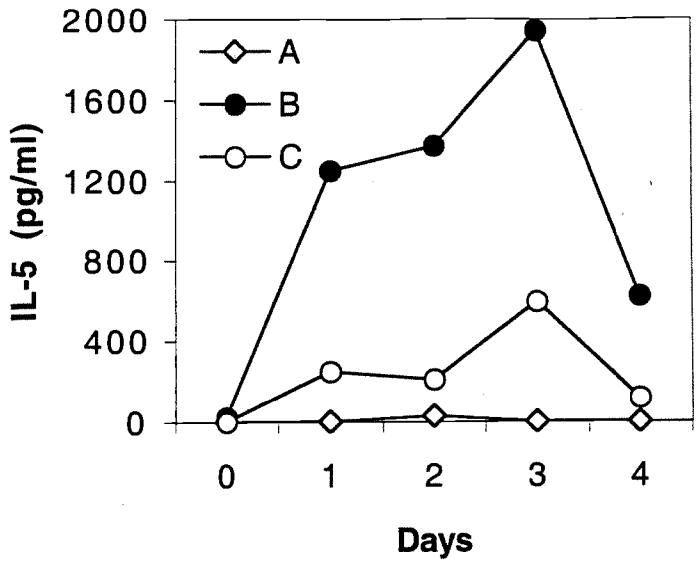
238 A BALF.

239 B

240 C


A
B
C
D
E
F
Total cell count (x104)
Total CD4+/OX40+ cs (x104)
2500
2000
1500
1000
500
0
100
80
60
40
20
6250
5000
3750
2500
1250
200
160
120
80
40
1250
1000
750
500
250
40
32
24
16
8
0 1 2 3 4
LN
Lung
BALF

IL-4	0	1	2	3	4
A	0	0	30	0	0
B	22	1245	1370.5	1943	622.5
C	0	248.5	209.5	596	116.5



IL-4	0	1	2	3	4
A	0	0	0	0	0
B	0	0	82	60	52
C	0	0	14	16	15